Arachidonic acid modulation of α1H, a cloned human T-type calcium channel

YI ZHANG, LEANNE L. CRIBBS, AND JONATHAN SATIN

Department of Physiology, The University of Kentucky College of Medicine, Lexington, Kentucky 40536-0298; and Department of Physiology and Cardiovascular Institute, Loyola University Medical Center, Maywood, Illinois 60153

Arachidonic acid modulation of α1H, a cloned human T-type calcium channel. Am. J. Physiol. Heart Circ. Physiol. 278: H184–H193, 2000.—Arachidonic acid (AA) and the products of its metabolism are central mediators of changes in cellular excitability. We show that the recently cloned and expressed T-type or low-voltage-activated Ca channel, α1H, is modulated by external AA. AA (10 µM) causes a slow, time-dependent attenuation of α1H current. At a holding potential of −80 mV, 10 µM AA reduces peak inward α1H current by 15% in 15 min and 70% in 30 min and shifts the steady-state inactivation curve −25 mV. AA inhibition was not affected by applying the cyclooxygenase inhibitor indomethacin or the lipooxygenase inhibitor nordihydroguaiaretic acid. The epoxygenase inhibitor octadecynoic acid partially antagonized AA attenuation of α1H. The epoxygenase metabolite epoxyeicosatrienoic acid (8,9-EET) mimicked the inhibitory effect of AA on α1H peak current. A protein kinase C (PKC)-specific inhibitor (peptide fragment 19–36) only partially antagonized the AA-induced reduction of peak α1H current and the shift of the steady-state inactivation curve but had no effect on 8,9-EET-induced attenuation of current. In contrast, PKA has no role in the modulation of α1H. These results suggest that AA attenuation and shift of α1H may be mediated directly by AA. The heterologous expression of T-type Ca channels allows us to study for the first time properties of this important class of ion channel in isolation. There is a significant overlap of the steady-state activation and inactivation curves, which implies a substantial window current. The selective shift of the steady-state inactivation curve by AA reduces peak Ca current and eliminates the window current. We conclude that AA may partly mediate physiological effects such as vasodilatation via the attenuation of T-type Ca channel current and the elimination of a T-type channel steady window current.

low-voltage-activated calcium channel; epoxyeicosatrienoic acid; cardiac; window current

THE T-TYPE CALCIUM CURRENTS share the common defining characteristic of a low-voltage activation range (reviewed in Ref. 49). In the cardiovascular system, T-type Ca currents (I_T) are present in pacemaking (15), conducting (21, 43), atrial (2, 3, 54), normal (10, 31), and hypertrophied ventricular myocardium (33). Although the macroscopic T-type current is smaller in amplitude and shorter in duration than the L-type current, the T-type current may contribute to the regulation of excitation-contraction coupling in the ventricle, particularly over relatively long intervals (42). The voltage-activation range of the T-type channel suggests an important role in mediating Ca entry in vascular smooth muscle as well.

Arachidonic acid (AA) disrupts calcium dynamics in neonatal rat cardiac myocytes (22). In native preparations, both L- and T-type channels are modulated by AA (38). AA and AA metabolites are important messengers in cell physiology and pathophysiology. The cytochrome P-450 metabolites of AA, such as the epoxyeicosatrienoic acids (EETs), are candidates for being endothelin-derivated hyperpolarizing factors (EDHFs; Ref. 35). In pathological conditions AA is elevated in response to ischemic episodes (46), and in normal physiological circumstances AA and epoxygenase metabolites of AA mediate hyperpolarization-induced vasodilation of human resistance arterioles (28). These results suggest a possible interaction between AA and Ca channels given the simplistic scheme that Ca channel blockade (or current attenuation) may contribute to reduced Ca entry and hyperpolarization. In this respect, AA and AA metabolite modulation of L-type Ca current are better studied than the T-type currents. Although the mechanism of AA and AA metabolite modulation of L-type calcium current is controversial, it is probably mediated secondarily via effects on channel phosphorylation. Xiao et al. (51) suggest AA epoxygenase metabolite-mediated elevation of cAMP, whereas Petit-JJacques and Hartzell (34) argue for AA-induced stimulation of a phosphatase.

α1H heterologous stable expression in HEK 293 cells allows us to study human T-type channel properties in a human cell and in isolation from L-type channels. T- and L-type channels are often expressed in the same cell. The overlap of activation ranges for T- and L-type currents complicates the detailed study of T-type current in native cells. The heterologously expressed T-type Ca channels reproduce functional properties measured in native mammalian cells. Central to this study is the similarity of α1H voltage dependence and kinetics to that of native cells. α1H is a voltage-gated ion channel cloned (9) with functional properties unique to T-type current, namely, a low-voltage range of activation, a < 10 pS conductance with no preference for Ba over Ca, and a crossover of decaying macroscopic...
currents for current-voltage protocols (reviewed in Ref. 49). Therefore, the main purpose of the present study was to determine the effects of AA specifically on \(I_T\).

**METHODS**

The \(\alpha 1H\) cDNA in the vector pCDNA3 was used to establish a stably transfected HEK 293 cell line (7). Briefly, 1 \(\times 10^6\) HEK 293 cells were transfected with 10 \(\mu g\) of \(\alpha 1H\) plasmid using Lipofectamine (GIBCO-BRL, Gaithersburg, MD), according to the manufacturer's protocol. Forty-eight hours posttransfection, the cells were passed onto 10 100-mm plates and 1.0 mg/ml G418 was added. After 10–14 days of selection, single colonies were isolated and expanded. The resulting stable transfectants were screened for T-type Ca\(^{2+}\) currents. The clonal line designated “HEK \(\alpha 1H\) -13” was selected for further studies. Cells are maintained in DMEM, 10% fetal bovine serum, with 100 U/ml penicillin, 100 mg/ml streptomycin (GIBCO-BRL), and 1.0 mg/ml G-418 (Mediatech). Immediately before experiments, culture medium was replaced with the whole cell bath recording solution consisting of (in mM) 140 NaCl, 5 CsCl, 2.5 KCl, 10 TEA-Cl, 2.5 CaCl\(_2\), 1 MgCl\(_2\), 5 HEPES, and 5 glucose, with a pH of 7.4. The pipette contained the following (in mM): 110 K-glucurate, 40 CsCl, 1 MgCl\(_2\), 3 EGTA, and 5 HEPES, pH 7.35 with CsOH. Experiments were performed in the presence of 10 \(\mu M\) TTX at room temperature (20–22°C).

We transiently transfected the human cardiac Na channel (\(\alpha\)1H, supplied by Dr. H. A. Hartmann, Baylor College of Medicine) into the HEK \(\alpha 1H\) -13-stable transformed cell line in some experiments. For the transient transfection protocol, HEK \(\alpha 1H\) -13 cells were grown to \(\sim 50\%\) confluence on 35 \(\times\) 10-mm cell culture plates. \(\alpha 1H\) plasmid cDNA (2 \(\mu g\)) was used for transfection using the calcium phosphate method. To identify \(\alpha 1H\) cells transiently coexpressing \(\alpha 1H\) Na channels, we cotransfected HEK \(\alpha 1H\) -13 cells with 2 \(\mu g\) of cDNA encoding green fluorescent protein (GFP). Cells were used 48–72 h after \(\alpha 1H\) + GFP cotransfection.

All voltage-clamp recordings were performed in the whole cell configuration of the patch-clamp technique. Current was digitized at 20 kHz (Digidata 1200C A/D board, Axon Instruments, Burlingame, CA) and low-pass filtered at 10 kHz. An Axon Instruments 200B amplifier (Axon Instruments) was used to record currents; series resistance compensation to 80% was employed. Data acquisition and analysis was performed with pCLAMP6 (Axon Instruments) and Origins 4.1 software (Microcal Software). Steady-state activation and inactivation curves were fitted to Boltzmann distributions. For the activation curve (conductance-voltage curve), peak current was plotted as a function of voltage and fit to a Boltzmann distribution with the reversal potential allowed to float. Plots were fitted to Boltzmann distributions. For the activation curve, the peak current shown in Fig. 1B: AA attenuation of peak current is partially reversible. Peak current declines progressively during 30 min of AA application. Washout of AA results in a slow return toward pre-AA control levels. However, washout of AA effects on peak current are not complete in 68 min of recordings. Interpulse interval, 20 s.

**RESULTS**

AA modulates \(\alpha 1H\), a cloned human T-type Ca channel. Figure 1 shows the inhibitory effect of AA on \(\alpha 1H\) current. Peak inward \(\alpha 1H\) current is inhibited 67% after 25 min of continual exposure to AA (Fig. 1A). Inward current was elicited by a voltage step to \(-20\) mV. To completely remove inactivation, we prepulsed the cell to \(-120\) mV for 5 s. The control recordings were initiated 10 min after patch rupture. At the time...
indicated in Fig. 1B, 10 µM AA was continuously applied for 30 min before washout. Figure 1B shows two salient features of AA modulation of α1H: a slow time course for the onset of AA-induced peak current attenuation and reversibility. Return to control bath solution resulted in a slow return of peak α1H current. AA also causes a speeding of the decay of macroscopic α1H current. The decay phase of current in Fig. 1A is fit by a single-exponential function. In this cell, AA speeds the time constant of decay from 24 to 14 ms. Washout of AA partially restores the decay time (18 ms) similar to the peak current response. In summary, both wash in and wash out of AA attenuation of peak α1H current occurs with a time course on the order of minutes.

The speeding of current decay by AA suggests interactions with the inactivation process of α1H. A possible

---

**Fig. 2.** AA shifts steady-state inactivation curve without a corresponding effect on activation curve. A: activation curve. Control (left); 20 min after addition of 10 µM AA (right). Current traces were elicited by step depolarizations ranging from −90 to +70 mV in 5 mV increments under control conditions. Note signature crossover of current decay characteristic of native and cloned T-type Ca channels. Currents were recorded in physiological Na and Ca concentrations. Reversal potential obtained from fitting current-voltage curve to a Boltzmann distribution was +49 mV. B: inactivation curve. Current traces in control conditions were elicited by a step to −20 mV following a 5-s prepulse to potentials ranging from −120 to −30 by 10 mV increments. C: conductance-voltage curves drawn from raw data of same cell shown in A (activation) and B (inactivation) before and 20 min after addition of 10 µM AA. In this example AA shifts the inactivation midpoint (V_{1/2}) by −27 mV. Activation and inactivation (macroscopic conductance as a function of voltage [G]) curves were drawn from peak currents in A and normalized to maximal conductance. For inactivation, a 5-s conditioning pulse was used to attain steady-state conditions. SSI, steady-state inactivation; SSA, steady-state activation; V_{test} or V_{condn}, test potentials. D: enlargement of C shows that AA eliminates a possible α1H window current (shaded area). E: voltage dependence of macroscopic current decay was fitted to a single-exponential function for all test potentials. Plot of time constant of current decay τ_{decay} vs. test potential for control and 10 µM AA. Data were pooled (means ± SE). Solid line is fit of exponential voltage dependence of time constants with an offset. Voltage-independent offset is 17.3 and 7.2 ms in control and AA, respectively. Voltage dependence of time constants were e-fold change per 7 and 19 mV for control and AA, respectively.
mechanism for the speeding of the decay of α1H current is a shift of the steady-state inactivation voltage dependence. To test this hypothesis we measured the steady-state voltage dependencies of α1H in control and in the presence of AA for 20 min. The steady-state activation and inactivation curves for α1H overlap in the narrow range of voltage from −55 to −45 mV. Figure 2A shows a family of whole cell currents elicited by graded depolarization in control and after 10 µM AA. As with native T-type currents and with Na channels, albeit with a slower time course, the α1H current displays a crossover during the decaying phase. The decaying phase of the current is fit well with a single-exponential function. For test potentials positive to −30 mV there is no dependence of decay with voltage (Fig. 2E). This is similar to the native T-current (e.g., Ref. 5), where the voltage-independent range can be represented as voltage-independent transitions among a closed, opened, and inactivated state (5). AA speeds the voltage-independent decay of macroscopic current approximately twofold. Therefore, the apparent speeding of decay induced by AA at −20 mV (Fig. 1) cannot be secondary to a hyperpolarizing shift of the current-voltage relationship (I-V) curve on the voltage axis. For depolarizations negative to −40 mV there is a steep dependence of macroscopic decay with voltage. AA dramatically increases the decay rate in the voltage-dependent range, resulting in a less steep dependence of decay on voltage.

The conductance-voltage curve drawn from the slope conductance of the peak I-V relationship in Fig. 2A shows the T-type defining characteristic low-voltage range of activation. The inactivation curve was obtained from peak currents elicited by a test depolarization to −20 mV preceded by 5-s conditioning pulses to −120 mV to remove inactivation. Figure 2C shows that the superimposition of the activation and inactivation curves results in a narrow range of voltage where channels are partially activated but not completely inactivated. This range of voltage represents a possible window current. AA (10 µM) selectively shifts the midpoint of the inactivation curve from −65.0 ± 1.4 mV in control to −88.9 ± 2.4 mV in AA (P < 0.001), without a concomitant shift of the activation curve. AA also reduces the slope of the peak activation curve from 5.4 ± 0.3 in control to 6.3 ± 0.1 with AA (P < 0.01). In the representative cell shown in Fig. 2, AA shifts the midpoint of inactivation (V1/2) by −27 mV in 20 min. The average shift of the inactivation V1/2 was −24 ± 1.6 mV (n = 7). The AA-induced shift is significantly greater than time-matched, drug-free control cells (n = 6). In the absence of added AA or with the addition of carrier only (DMSO), we measured an average shift of −3.8 ± 1.4 mV in 20 min. It is important to show reversibility to unequivocally demonstrate that the hyperpolarizing shift following AA is not an artifact, despite the significant difference of inactivation V1/2 between control versus AA treatment. Figure 3 shows that following 50 min of washout, both maximal conductance (Gmax) and V1/2 are reversible. Although AA renders activation a less steep function of voltage, it has no effect on the steady-state activation curve midpoint (control V1/2 = −42.1 ± 1.3; AA V1/2 = −42.8 ± 1.5 mV). Consequently, the overlapping window of inactivation and activation is eliminated (Fig. 2D).

Cytochrome P-450 metabolites modulate α1H. The metabolism of AA occurs via three principal pathways: cyclooxygenase, lipoxygenase, and epoxygenase catalysis. To test whether one or more of these AA metabolic pathways mediates AA effects on α1H, we coadministered inhibitors with AA. Neither 10 µM NDGA nor 10 µM indomethacin prevented the AA-induced attenuation of α1H current. These results are summarized in Fig. 4. In contrast, the cytochrome P-450 suicide substrate inhibitor 17-ODYA only partially antagonized AA attenuation of α1H current. 17-ODYA by itself has no significant effect on α1H current; however, cells preincubated in 17-ODYA for 20 min before recording show only partial modulatory effects of AA. In the presence of 17-ODYA, peak current is attenuated 23% and the V1/2 is shifted by −12.2 ± 2.5 mV (n = 5; Fig. 4). To test for a direct interaction, we used the nonmetabolizable analog of AA, ETYA. A 10 µM concentration of ETYA caused a statistically significant attenuation of peak α1H
Fig. 4. AA modulates α1H channels via direct interaction and epoxygenase metabolites. Lipoxigenase and cyclooxygenase metabolites do not contribute to AA modulation of α1H in HEK 293 cells. For all cases we initiated control recordings 10 min after patch rupture. A–C compare parameters obtained from control (immediately before drug addition) with those from 20 min after drug addition. A: pooled peak current attenuation by 20 min of exposure to 10 µM of AA, AA analogs, or AA metabolic inhibitors. ***P < 0.001, **P < 0.01 vs. time-dependent control. ##P < 0.001 vs. AA; and $P < 0.05 vs. octadecynoic acid (17-ODYA). B: steady-state V1/2 is shifted in hyperpolarized direction by AA. Although 10 µM 17-ODYA partially antagonizes AA-induced shift, 10 µM ETYA has no effect. **P < 0.01 and *P < 0.01 vs. time-dependent control. ##P < 0.001 vs. AA. $*$P < 0.001 vs. 17-ODYA + AA. C: AA has no effect on voltage dependence of steady-state activation. NDGA, nordihydroguaiaretic acid.

Fig. 5. Epoxyeicosatrienoic acid (8,9-EET), an epoxygenase metabolite of AA, partially mimics inhibitory effect of AA on α1H currents. A: peak current attenuation by 20 min of exposure to 10 µM AA, 100 nM 8,9-EET, or vehicle (for time-dependent control). 17-ODYA does not inhibit 8,9-EET attenuation of macroscopic Gmax. B: 8,9-EET has no effect on V1/2 for steady-state inactivation of α1H. V1/2 values for steady-state inactivation before and after addition of 8,9-EET are −66.0 ± 1.76 and −68.6 ± 3.69 mV, respectively. *P < 0.001 vs. time-dependent control. ##P < 0.01 vs. AA. ###P < 0.001 vs. AA.

current (P < 0.05) and a slight but statistically insignificant hyperpolarized shift of the inactivation curve.

The EETs are candidates for EDHF (4, 18 but see Refs. 11 and 48). It was surprising that cytochrome P-450 inhibition partially antagonized the response of α1H current to AA because it is often noted that cells in culture downregulate cytochrome P-450 expression (7, 17). Therefore, we directly tested whether 8,9-EET, a specific cytochrome P-450 metabolite, modulates α1H.

Figure 5 compares the effects of 10 µM AA with 0.1 µM 8,9-EET added to the bath solution. 8,9-EET causes a 31% reduction of Gmax. This is about one-half that obtained with AA alone. In contrast to the AA modulation of α1H, the inactivation V1/2 is not shifted by 8,9-EET. To control for a possible nonspecific 17-ODYA effect on Iα, we also tested whether 17-ODYA altered the 8,9-EET modulation of α1H current. Figure 5 shows that 17-ODYA does not inhibit the 8,9-EET inhibition of Gmax. Therefore, we conclude that either AA or the AA epoxygenase metabolite 8,9-EET can modulate α1H conductance.

PKC, but not protein kinase A, mediates AA modulation of α1H. The slow time course of AA and AA metabolite modulation of α1H current argue for a role for intracellular intermediates. Although AA modulation of L-type channels is well studied, the mechanism...
of action is controversial. It is likely that AA modulation of L-type Ca channels occurs via effects on cAMP and, in turn, protein kinase A (PKA) (51) or phosphatase intermediates (34). We performed three experiments that established the lack of an effect by PKA on a
erg 1H current: 1) addition of 8-BrcAMP; 2) phosphatase inhibition by okadaic acid (OA); and 3) AA modulation in the presence of PKA-inhibitor peptide. Addition of the membrane-permeable, nonhyrdrolyzable form of cAMP, 8-BrcAMP, as high as 3 mM has no effect on the inhibitory modulation of the a
1H current (Fig. 6A).

Despite chronic elevation of the nonhydrolyzable 8-BrcAMP, 10 µM AA still induced attenuation of peak a
1H current (Fig. 6A). The effects of AA on I
T are the same with or without 8-BrcAMP. If the channel is basally maximally phosphorylated, this could be a false negative result; therefore, we tested the effect of the phosphatase 1 and 2a inhibitor OA. Figure 6B shows that OA has no effect on I
T consistent with the interpretation that the a
1H channel is not basally phosphorylated at a phosphatase 1- or 2A-sensitive site. Note that OA blocks dephosphorylation of L-type channels (14, 20, 34, 39). To eliminate any possible contribution by PKA to AA modulation of I
T, we perfused cells intracellularly with 30 µM PKA inhibitor peptide. PKA inhibitor had no effect on AA modulation of I
T (Fig. 6B), establishing that under our recording conditions a
1H is not basally phosphorylated by PKA.

To control for the possibility that HEK 293 cells lack PKA activity following stimulation by 8-BrcAMP, we transfected a
1H-stable transfected cells with the human cardiac voltage-gated Na channel hHla. Cardiac Na current is modulated by cAMP and therefore was used as an assay for 8-BrcAMP modulation. Figure 6C shows that OA and PKA inhibitor peptide do not affect the responses of a
1H to 8-BrcAMP.

Figure 6B shows that OA and PKA inhibitor peptide do not affect the responses of a
1H to 8-BrcAMP.
shows selected current sweeps elicited at −40 mV. In cardiac myocytes, cAMP analogs cause a shift of the peak activation curve for the voltage-gated Na channel in the hyperpolarized direction. Similarly, 8-BrcAMP shifts the hH1a peak activation curve. The selective effect of such a shift is manifested as an increase of peak current at more hyperpolarized potentials. The presence of α1H T-channels and hH1a Na channel current is evident from the fast and slow inward currents at −40 mV in the presence of 8-BrcAMP (Fig. 6C, middle trace). To confirm that the fast inward current is caused by Na channels, we blocked the shift effect with 30 µM TTX (Fig. 6C, right trace and I-V curve). Note that the late inward current is unaffected by 8-BrcAMP. We therefore conclude that AA modulation of the human T-type channel α1H is not via a PKA pathway under our recording conditions.

PKC activation is one of many downstream events following elevation of AA. Therefore, it is reasonable to test whether AA mediates effects on I_{1T} via PKC activation. We assessed AA effects in the presence of PKC inhibitor peptide in the intracellular pipette solution. Inclusion of 100 µM PKC inhibitor peptide without drug addition has no effect on α1H current. This suggests that the T-channel in our expression system is not basally modulated by PKC. However, Fig. 7 shows that in the presence of 100 µM PKC inhibitor peptide the AA modulation of I_{1T} is only partially antagonized. Under the condition of PKC inhibition AA decreases G_{max} and shifts the V_{1/2} significantly less than when PKC is active. Under our recording conditions we buffer bulk Ca concentration to nanomolar levels with EGTA and dialyze the cytosol for at least 10 min. Therefore, our data suggest that AA modulates I_{1T}, but only in part, via a Ca-independent PKC that is membrane associated.

PKC inhibition only partially antagonizes the AA-induced shift of V_{1/2} and decrease of I_{1T}. In contrast, 8,9-EET only reduces G_{max}. To test whether PKC is an intermediate in the G_{max} reduction by 8,9-EET, we tested the effect of 8,9-EET in the presence of 100 µM PKC inhibitor peptide. PKC inhibitor peptide has no significant effect on the 8,9-EET reduction of G_{max} (Fig. 7), consistent with a direct 8,9-EET modulation of I_{1T}.

**DISCUSSION**

This is the first demonstration that the cloned T-type channel derived from human heart α1H is reversibly modulated by AA. The present study was motivated by the clinical finding that drugs that block T-type Ca channels relieve hypertension (19). AA and in particular cytochrome P-450 metabolites of AA may contribute to vasorelaxation (17); therefore, it is reasonable to evaluate whether AA and cytochrome P-450 metabolites of AA block I_{1T}. In this study we show that AA and the AA metabolite 8,9-EET reduce Ca conductance through the α1H T-type Ca channel. In addition AA causes a selective shift of the steady-state inactivation curve. This selective shift of the inactivation curve eliminates a possible window current. AA modulation of α1H is not via a PKA intermediate, but AA effects are partially attenuated by either PKC blockade or preincubation with 17-ODYA. In contrast, the 8,9-EET attenuation of I_{1T} is independent of PKC.

AA modulation occurs slowly with a time course over the range of minutes in our study. It is unlikely that the slow time course of AA modulation of α1H reflects partitioning of AA into the membrane, because AA rapidly incorporates into and flip-flops across the lipid bilayer (50). If the slow time course was due to AA metabolism, then we would have expected to observe a more rapid effect with either nonmetabolizable AA analogs or with specific AA metabolites. Direct application of the cytochrome P-450 metabolite 8,9-EET also modulates α1H with a slow time course. AA and EET
effects on Ca current are either mediated by membrane-associated intermediates or further metabolized. The majority of EETs are esterified to cellular glycerophospholipid (25). Interestingly, the time course of coenzyme A/ATP-dependent metabolism of EET to either EET-PC or EET-PI occurs on a time scale of minutes (25). Furthermore, Chen et al. (6) recently reported a direct modulation of L-type Ca channels by EET-phospholipids. Interestingly, in agreement with our T-channel studies, EET reduces L-type open-channel probability ($G_{\text{max}}$) and speeds inactivation presumably by a direct EET-phospholipid-channel mechanism (6).

Although PKA consensus sites exist on the $\alpha$H1 channel, our results show that they are not used for channel modulation. Similarly, early reports of native cardiac I_T show no evidence for PKA modulation (45). This negative result removes a source of complexity for interpreting the pathway of the AA modulation of T-type current. In contrast to AA modulation of closely related L-type channels, there is no role for PKA as an intermediate in the modulation of T-type Ca channels.

Our observation that AA induces a $-25$ mV shift of inactivation and a reduction of $G_{\text{max}}$ whereas EET only causes a reduction of $G_{\text{max}}$ argues for possibly separate mechanisms of action. We cautiously interpret the finding that PKC inhibitor peptide and 17-ODYA inhibit both the inactivation shift and the $G_{\text{max}}$ attenuation. First, the inhibitory effect, though significant, is only a partial effect. Second, we were unable to positively identify a cytochrome P-450 isoform in our cells. Note, however, that with over 200 different cDNAs encoding cytochrome P-450 isoforms (reviewed by Ref. 17), it is very difficult to unequivocally prove the absence of cytochrome P-450. Nevertheless, the cytochrome P-450 enzyme family is inducible and is downregulated in tissue-cultured cells (7, 17). Third, NADPH is the reductant in cytochrome P-450-mediated epoxidation of AA; however, we did not include NADPH in the pipette. It is interesting to note that 17-ODYA affected K channels in freshly isolated portal vein recorded in the whole cell mode without the addition of NADPH (12). It is possible that intracellular dialysis through the whole cell pipette is incomplete. 17-ODYA is a cytochrome P-450 suicide substrate inhibitor (55), and there are no reports of 17-ODYA side effects. However, 17-ODYA and ETYA are structurally similar to AA and may be competing for AA reincorporation into the membrane or a common fatty acid binding site. This suggests that AA metabolic inhibitors are weak agonists that are acting as competitive inhibitors for an AA receptor site or fatty acid binding site.

Two established downstream effects of AA include PKC activation (30, 32, 41) and small G protein activation via inhibition of small GTPase activating protein (16, 23, 40). Whereas there are two reports of Ras modulation of L-type Ca channels (13, 24), there are no known studies of Ras-T-type channel interactions. Our partial PKC inhibitor results are similar to those observed for neuronal L-type currents (26) and suggest that AA activation of PKC may lead to PKC-channel interaction. Surprisingly, however, there are few studies of PKC modulation of T-type Ca channels despite the central importance of PKC and Ca currents. In heart cells, Tseng and Boyden (44) showed that native I_T is attenuated following activation of PKC. There are several PKC consensus sites on $\alpha$H1, two sites on the domain I-II cytoplasmic connector and one site on the domain III-IV connector (9). Whereas a consensus site is a prerequisite for PKC modulation, its presence does not mean that the channel is a substrate. This adage is illustrated by the absence of PKA modulation of $\alpha$H1. Nevertheless, independent evaluation of PKC modulation of T-type channels is an important area for further study.

We expressed effects on steady-state activation based on peak current. However, the peak current may be contaminated by overlapping activation and inactivation transitions, particularly in the low-voltage activation range (10). Nonetheless, there is important biophysical information to be gleaned from the current-voltage protocols used in this study. Our data suggest that AA speeds a voltage-independent open-to-inactivation transition, and AA also alters a voltage-dependent transition. The macroscopic current inactivation is well fit by a single-exponential function over the entire voltage range (Fig. 2). Macroscopic decay is voltage-dependent for weak depolarization; however, for strong depolarizations (greater than $-30$ mV), the macroscopic inactivation decay rate as a function of voltage [$\tau (V)$] is constant. Our $\tau (V)$ curve for macroscopic decay is similar to that reported from native I_T preparations (5, 8). As Chen and Hess (5) point out, this extreme potential-limiting rate suggests a voltage-independent open-to-inactivated state transition. The speeding of the rate-limiting decay implies that AA modulates channel inactivation. The more dramatic depression of the voltage dependence by AA of the $\tau (V)$ curve is more complicated, and the explanation depends on the kinetic scheme. For $\alpha$H1, as for native T-channels (5), Na channels (1), and some K channels (53), it is a good assumption that only the initial activation transitions are voltage dependent. AA reduces both the macroscopic inactivation voltage dependence and the slope of the activation curve. With a minimal number of assumptions, our results imply that AA exerts its effect mainly on a voltage-dependent, closed-state transition.

The low-voltage-activation range of T-type Ca channels suggests that its principal function is to mediate the upstroke of the action potential. In pacemaker tissues, such as nodal cells, blockade of T-type current slows spontaneous firing (27). AA and its metabolites have long been known to induce bradycardia (29). Thus it is reasonable that AA may mediate bradycardia at least in part via blockade of T-type channels. Our Na channel coexpression experiment is a side issue in this paper; nonetheless, the overlapping range of activation of Na and T-type currents illustrates the important point that either of these current carriers activate in a range that enables them to carry the upstroke of the action potential. Although T-type Ca channels are not normally present in the working myocardium, cardiac
hypertrophy induces de novo expression of T-type channels (33). Under pathophysiological conditions, AA or EETs may have an important protective function. For example, hypertrophic and ischemic myocardium slow reentry pathways can lead to ventricular fibrillation and in turn sudden cardiac death. During ischemia the extracellular space is acidified, and the maximum diastolic potential (MDP) modestly depolarizes (47, 52). The MDP is in a range that is conducive for T-channel activation but is still rather hyperpolarized for L-type Ca channels.

The antihypertensive efficacy of the relatively specific T-type channel blocker mibefradil (9, 37) suggests a role for T-type channels in control of vascular tone. There is evidence that the EDHF is one or more specific T-type channel blocker mibefradil (9, 37) suggests a mechanism for inhibition of ectopic action potential initiation.

In conclusion, AA modulation of T-type Ca channels reduces channel current via both a PKC-dependent and -independent pathway. In contrast, 8,9-EET reduction of T-type current may be caused by a direct interaction with the channel.

We thank Brian Delisle and Edward Perez-Reyes for insightful discussions and comments on this manuscript. We are grateful to Alison Nemes for excellent technical support and to Brian Jackson for performing RT-PCR assays for several cytochrome P-450 isoforms in our cells. We also thank Robert Rosenberg for alerting us to the publication of similar EET effects on L-type Ca channels (Mol. Pharmacol. 55: 288–295, 1999).

Address for reprint requests and other correspondence: J. Satin, Dept. of Physiology, MS-508, University of Kentucky College of Medicine, 800 Rose St., Lexington, KY 40536-0298 (E-mail: jsatin1@pop.uky.edu).

Received 12 February 1999; accepted in final form 23 July 1999.

REFERENCES


